# Efficiency of High-Pressure Treatment on the Inactivation of *Escherichia coli* O157:H7 in Tomato Juice and Liquid Whole Egg.

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## ABSTRACT

Liquid foods and beverages have been implicated in numerous food-borne outbreaks and recalls. In order to preserve and reduce *E. coli* 0157:H7 population of tomato Juice and liquid whole egg, the efficacy of a non-thermal high-pressure treatment with a moderate temperature was investigated. Also, survival and viability loss of the *E. coli* 0157:H7 in tomato Juice and liquid whole egg was investigated. Tomato Juice and liquid whole egg were individually inoculated with *E. coli* 0157:H7 at $10^8$ cfu/ml and then exposed to continuous or repeated cycles of high-pressure treatment at a moderate temperature. Pressure at 300MPa, 350MPa and 400 MPa for up to 60 min reduced the population of inoculated *E. coli* O157:H7 by approximately 3.0, 3.0 and 5.0 log cycles, respectively in tomato juice. Population of *E. coli* O157:H7 in all samples tested was decreased when temperature was increased from 30-50°C. However, increases in temperature with a 600MPa resulted in coagulation of liquid eggs. Also higher numbers of injured cells were recovered in treated tomato juice than liquid egg. Repeating (4 times) the pressure at 300 MPa for a total of 10 min at room temperature was significant ($p<0.05$) in reducing *E. coli* population by extra 1 log. The results of this study suggests that repeating high-pressure treatment at least 4 times at 300 MPa for a total of 10 min would inactivate more *E. coli* O157:H7 strains in liquid whole egg, tomato juice and than using continuous pressure for the same amount of time.

## INTRODUCTION

High-hydrostatic pressure is a promising alternative to heat pasteurization for preservation of minimally processed foods because of its capacity to inactivate pathogenic microorganisms with minimal heat treatment, resulting in the almost complete retention of nutritional and sensory characteristics of fresh food without sacrificing shelf life. (*16*). For the last 15 years, the use of HPP has been explored extensively in food industry and related research institutions due to the increased demand by consumers for improved nutritional and sensory characteristics of food without loss of "fresh" taste. In recent years, HPP has been extensively used in Japan and a variety of food products like jams and fruit-juices have been processed (*9*).

*Escherichia coli* O157:H7 has emerged with increasing frequency in the past decade as an important food-borne pathogen causing hemolytic uremic syndrome (HUS) and hemolytic colitis (HC) in human beings (*13, 14*). Foods such as potato, turkey roll, yogurt, apple cider, raw milk, and raw fruits and vegetables are also source for this organism (*4, 15*). Recent outbreaks involving *Escherichia coli*

O157:H7 in apple (*5, 7*) and orange juices (*6*) has raised concerns about the safety of consuming unpasteurized fruit juices. The use of high-hydrostatic pressure at low pH has been shown to inactivate *E. coli* O157:H7 in fruit juices and other fruit products (*2, 10*). Garcia-Graells *et al.* (*8*) reported a 5-log decrease in the population of the surviving high-pressure-resistant mutants of *E. coli* in apple juice (pH 3.3) after treatment at 20$^0$C with 300 MPa pressure for 15 min. However, Linton *et al.* (*10*) showed a 6-log inactivation of *E. coli* O157: H7 in orange juice (pH 3.9) when treated with 550 MPa for 5 min at 20$^0$C.

The objective of this study was to determine an appropriate high hydrostatic pressure–temperature-time combinations potential for inactivating *E. coli* O157:H7 in tomato juices, and in liquid egg and also to investigate if repeating pressure treatment would achieve a better log reduction for *E. coli* O157:H7.

## MATERIALS AND METHODS

**Test strains.** Enterohemorrhagic *E. coli* O157:H7, strains CR-3, MN-28, MY-29, and DT-66 (isolated from bovine feces) were used in this study. Nalidixic acid–resistant strains of *E. coli* O157:H7 were prepared in our laboratory by chemical mutagenesis according to the method of Adelberg *et al.* (*1*). To minimize the contamination of the enumeration media by microorganisms that are naturally present on tomato juice or liquid eggs, all test strains of *E. coli* O157:H7 were adapted to grow in tryptic soy broth (TSB, pH 7.3; Nissui Seiyaku, Tokyo, Japan) supplemented with nalidixic acid (50 μg/ml).

**Preparation of inoculum.** Fresh overnight grown cultures (1 ml) approximately $10^9$ CFU/ml) were transferred to 99 ml of TSB medium containing nalidixic acid and incubated at 37$^0$C for 18h. After 18 h of incubation, cell suspensions were centrifuged at 3,000 x *g*, 10 min, 20$^0$C. Cell pellets were washed with equal volume (100 ml) of sterile phosphate-buffered saline (PBS, pH 7.2) solution. Finally, the washed cells were resuspended and concentrated to $10^9$ CFU/ml. The inoculum was maintained at 4$^0$C and applied to the test samples within 1 h of preparation.

**Inoculation and treatment procedures.** Phosphate buffered saline (10mM; pH 7.0) was prepared in the laboratory. Commercial, tomato juices (pH 4.2) were obtained from Kagome Co. Ltd. Tokyo, Japan. Fresh eggs from IFuji Dairy Industry (Ibaraki, Japan) were used in this study. Each egg was washed in warm soapy water (11$^0$C) with a brush after which the eggs were sanitized by immersion in ethanol (70%) for 10 min. The sanitized eggs were left to dry in a laminar airflow cabinet. The egg contents were aseptically removed by cracking into sterile bags and homogenized for 1 min in a stomacher. Cell suspensions of *E. coli* O157:H7 (1 ml) were inoculated into each PBS, tomato juice and liquid egg (9 ml) to give a final cell concentration of 5.0 x$10^8$ CFU/ml. Two milliliters of the inoculated PBS, tomato juices or liquid eggs was then dispensed into individual sterile plastic bags (5.0 by 8.0 cm) and sealed using a Doughboy heat-sealer. Individual plastic bags were double-bagged (7.0 by 10.0 cm) and sealed to prevent leakage of the contents during high-pressure treatment. All individually sealed plastic packages were kept at 4$^0$C prior to pressurization, which did not exceed 1 h. A batch hydrostatic pressurization unit (Model HYPREX R7K-3-15, Yamamoto Suiatsu Kogayosha Co. Ltd., Osaka, Japan) capable of operating up to 700 MPa with temperature range from 0 to 70$^0$C was used in this study. The rate of pressure increase was about 240 MPa/min, and pressure come-down time was less than 1 min. Pressurization time reported in this study did not include the come-up and come down times. The inoculated tomato juice and PBS were

pressurized at 200-600 MPa at ambient temperature for up to 60 min and Liquid whole egg were pressurized 300-400 MPa for up to 40-60min. In an experiment designed to study the effect of repeating pressure on the survival and inactivation of *E. coli* O157:H7, a repeating (four times) pressure of 300 MPa for 10 min at room temperature was also performed. Immediately after pressurization, the plastic bags were removed, cooled in an ice bath, and stored at 4°C prior to enumeration of CFU (within 2 h). Unpressurized cell suspensions were enumerated as controls.

**Enumeration of viable *E. coli* O157:H7.** Samples from pressurized PBS, tomato juices and liquid egg were serially diluted in buffered peptone water (pH 7.0, Difco Laboratories, Detroit, Mich.) and plated onto Sorbitol MacConkey agar (Nissui) containing 50μg/ml nalidixic acid (SMAN) and, tryptose soy agar (Nissui) containing 50μg/ml nalidixic acid (TSAN) for enumeration of *E. coli* O157:H7. All selective and non-selective agar plates were incubated at $37^0$C for 48 h to enhance recovery of pressure-injured cells. The number of CFU/ml of bacteria on nonselective and selective agar media was used to calculate the viability loss and injured survivors. Samples from positive and negative controls were plated onto agar media as mentioned above and incubated at similar temperatures for 48 h.

**Statistical analyses.** All experiments were done in triplicate with duplicate samples being analyzed at each sampling time. Data were subjected to analysis of variance (ANOVA) using Microsoft excel program. Significant differences in plate count data were established by least significant difference at the 5% level of significance.

## RESULTS AND DISCUSSION

The selection of *E. coli* O157:H7 strains CR-3, MN-28, MY-29, and DT-66, was investigated in 10 mM Phosphate buffer (pH 7.0) and the results showed that *E. coli* O157:H7 strain MN-28 was the most resistant strain when pressurized at 400 MPa for 10 min at ambient temperature (Table 1). There were considerable variations in pressure resistance among the *E. coli* O157:H7 strains of the same species, and in different food products. Our results were consistent with previous findings that reported differences in resistance of these pathogens to high-hydrostatic pressure (*3, 11*).

In our previous study, we reported that viability loss among the four *E. coli* O157:H7 strains studied ranged from 0.98 to 7.69 log cycles, with *E. coli* MN-28 being the most resistant strain under the conditions tested. The difference between the most resistant and most sensitive strains is about a 7.5-fold (Table 1). *E. coli* O157:H7 strains MN-28 which was the most resistant strain under the conditions tested, was used for the rest of the study.

TABLE 1. Viability loss of tested food-borne pathogens following pressurization at 400 MPa for 10 min at 25°C[a]

| *E. coli* 0157:H7 | $Log_{10}$ CFU/ml of Initial inoculum | $Log_{10}$ CFU/ml after treatment | Viability loss ($log_{10}$) |
|---|---|---|---|
| MN-28 | 8.44 ± 0.26 | 7.46 ± 0.19 | 0.98 ± 0.07 |
| CR3 | 8.32 ± 0.34 | 5.16 ± 0.23 | 3.16 ± 0.10 |
| DT-66 | 8.29 ± 0.16 | 3.54 ± 0.20 | 4.65 ± 0.08 |
| MN-29 | 8.34 ± 0.21 | 0.85 ± 0.10 | 7.69 ± 0.11 |

[a]N = 8. Values are means ± SD of three experiments with duplicate determinations per experiment.

The effect of HHP treatment on *E. coli* O157:H7 strain MN-28 in tomato juice is presented in Figure 1. Moderate pressure up to 400 MPa for 60 min at room temperature reduced the population by 5-6 log. Increased pressure up to 600 MPa for 15 min caused a significant ($p < 0.05$) viability loss of more than 8 log cycles for the selected pathogens in tomato juices studied. (Figure 1) However, a repeating (four times) cycle of pressure at 300 MPa for 10 min at room temperature resulted in similar log reduction of *E. coli* O157:H7 population in tomato juice (Figure 4b).

The high-pressure inactivation of *E. coli* O157:H7 strain MN-28 in Phosphate buffer is presented in Figure 2. Continious pressurization at 300MPa for 10 min caused a 1-1.5 log CFU/ml reduction of *E. coli* O157:H7; at 60 min of continuous pressurization at 300MPa an additional 1.0 log CFU/ml reduction was observed. Increasing the pressure resulted to an increase in inactivation rate, and at a pressure of 600 MPa for 15 min, a reduction of approximately 7.0 log CFU/ml was achieved (Figure 2). The proportion of injured cells resulting from the above treatment increased with increasing pressure. Repeating the pressure treatments resulted in a similar but to a lesser log reduction (4.0 log CFU/ml) in PBS than the populations in tomato juice. Also, higher population of injured cells was recovered in samples treated with repeated cycles of pressure (Figure 4).

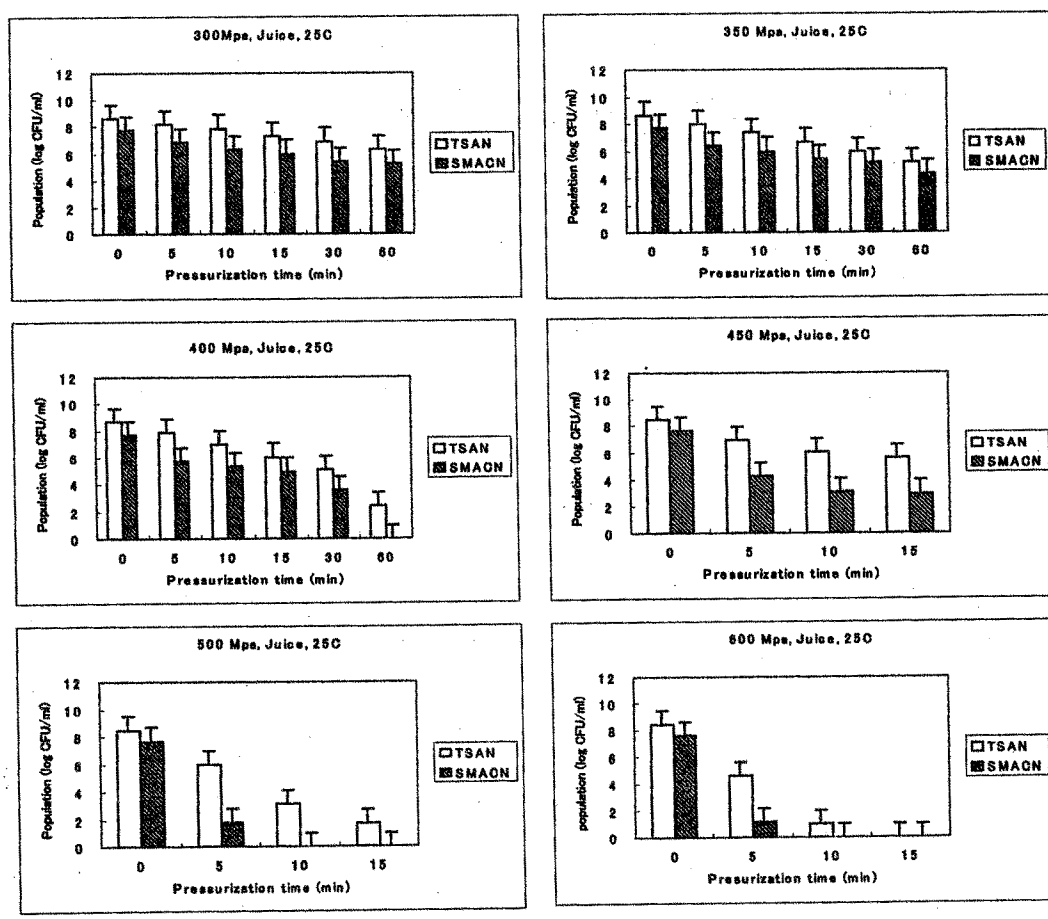


Figure 1. Inactivation of *Escherichia coli* O157:H7 inoculated in tomato juice by hydrostatic pressure at 300-600 MPa at ambient temperature. Values are means ± SD of three experiments with duplicate determinations per experiment.

Low pH enhances the inactivation of vegetative bacteria by high pressure while, the combination of low temperature storage and acid pH prevent outgrowths and all together resulted in further inactivation of cells that are sub-lethally injured by the pressure treatment (*9, 10*). In our experiment, injured population of *E. coli* O157:H7 recovered in PBS was greater than the numbers enumerated in the tomato juice. This particular observation may be attributed to the pH of the PBS which was capable of maintaining normal physiological and biochemical process. Similarly, the damage cells in tomato juice with a pH of 5.5 are unable to repair, hence their tolerance and survival at this pH was low.

When inoculated liquid whole egg was pressurized up to 400 MPa for 40 min at ambient temperature, a 4.0-log inactivation of *E. coli* O157:H7 strains was achieved (Figure 3). These results indicate that the treatment of 400 MPa for 40 min may not be sufficient to give a 6-log reduction of *E. coli* O157:H7 in liquid whole egg. However, repeating the cycles four times for a total of 10 min achieved a higher rate of inactivation than when a continuous treatment of equal time was used. The effect of continuous pressure at 400 MPa for 40 min was equivalent to a 3 repeats of cycles treatment at 300 MPa (Figure 4). Liquid whole egg is a sensitive product with high microbial population; a minimal treatment of 3 cycles with moderate heat may be appropriate for microbial reduction without sacrificing protein functionality. The treatment temperature and/or time may be increased slightly, because of the susceptibility of egg proteins to coagulation or thermal denaturation with the formation or destruction of covalent bonds, which may result in flavor changes and damage to the functional properties of the liquid whole egg.

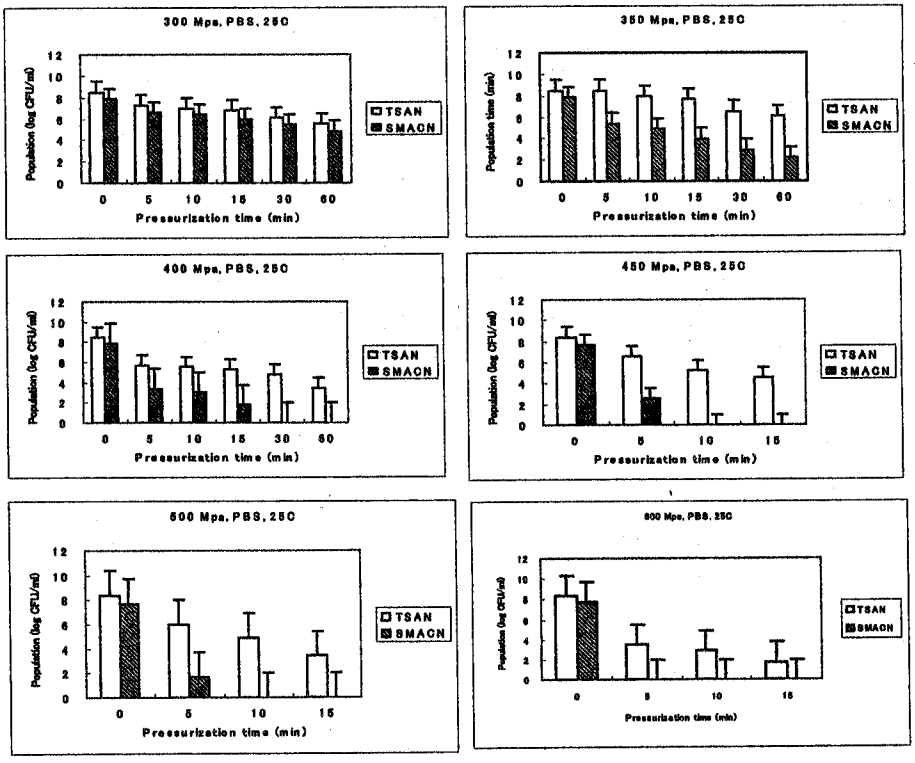


Figure 2. Inactivation of *Escherichia coli* O157:H7 inoculated in Phosphate buffered saline (pH 7.0) by hydrostatic pressure at 300-600 MPa at ambient temperature. Values are means ± SD of three experiments with duplicate determinations per experiment.

The proportion of injured cell in both continuous and repeating high-pressure treatment in liquid whole egg was less than that of tomato juice. The differences in survival and/ or viability loss observed in this study may be due to types of nutrient associated with each liquid food studied. It has been reported that fat, pH and other factor can confer protection against pressure, which may influence the viability of the microorganisms against the pressure treatment (*12*). Therefore, it is necessary to understand how quickly the microorganisms are inactivated depending on pressure, temperature, and their surroundings.

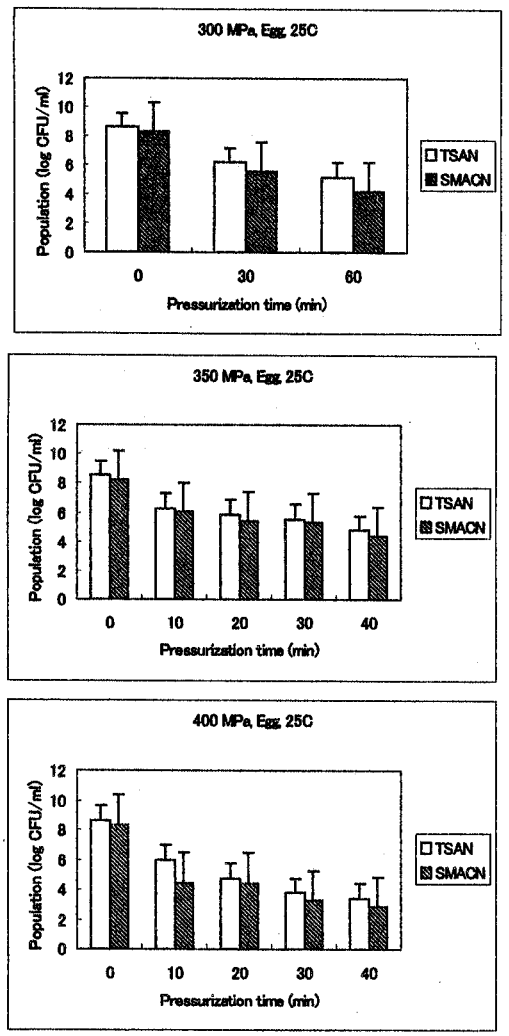


Figure 3. Inactivation of *Escherichia coli* O157:H7 inoculated in liquid whole egg by hydrostatic pressure at 300-400 MPa at ambient temperature. Values are means ± SD of three experiments with duplicate determinations per experiment.

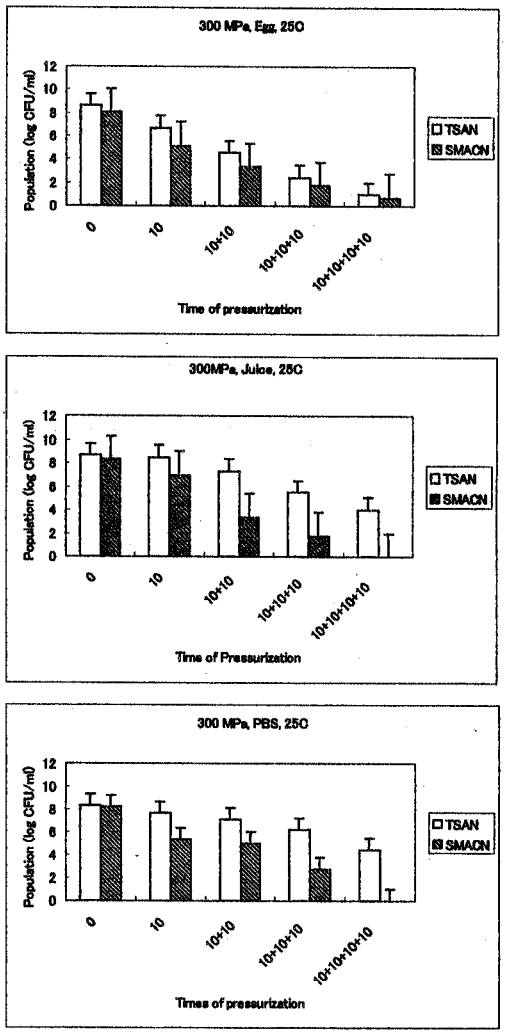


Figure 4. Inactivation of *Escherichia coli* O157:H7 inoculated in (a) Tomato juice, (b) PBS and (c) liquid whole egg by repeating hydrostatic pressure at 300 MPa at ambient temperature. Values are means ± SD of three experiments with duplicate determinations per experiment.

## CONCLUSIONS

Results of this study clearly showed that destruction of *E. coli* O157:H7 in tomato juice, PBS and in whole egg are dependent on pressure and the temperature at which it was applied, and the inactivation of the pathogen tested was less pronounced at continuous pressure while repeating pressure treatments gave better results. Treatment with the repeated pressure cycle with mild heating ($50^0$C) improved the efficacy of HPP for inactivating *E. coli* O157:H7 in liquid whole egg and tomato juice. Therefore, the results of this study suggest that repeating high hydrostatic pressure for four cycles would be an effective means of pasteurizing tomato juice and liquid whole egg to reduce microbial numbers and extend shelf life.

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